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Pulmonary Function and Bronchial Reactivity in Human Subjects with Exposure to Ozone and Respirable Sulfuric Acid Aerosol

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PULMONARY FUNCTION AND BRONCHIAL REACTIVITY IN HUMAN SUBJECTS WITH EXPOSURE TO OZONE AND RESPIRABLE SULFURIC ACID AEROSOL

AN ENVIRONMENTAL CHAMBER STUDY

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16. ABSTRACT

A three-year research study was conducted at the University of Maryland Environmental Chamber, investigating the effects of individual and sequential exposures to ozone and sulfuric acid aerosol on pulmonary function and bronchial reactivity in human subjects. PHASE I: In healthy smokers and nonsmokers exposed for 4 hours to 98 $\mu g/m^3$ 0.14 μm H_2SO_4 aerosol, no significant changes in pulmonary function (FVC, FEV_1, FEV_3, SGaw, FRC, SBNER, Phase IV, C_L stat, C_L dyn, R_L) were observed with exposure or 24 hours post-exposure. PHASE II: The effect of 0.3 ppm O_3 on a subsequent exposure to 100 $\mu g/m3$, 0.13 μm H_2SO_4 aerosol was studied in nonsmoking subjects to determine if pre-exposure to O_3 would sensitize these individuals to H_2SO_4 aerosol. Two-hour O_3 and 4-hour H_2SO_4 exposures were employed. Pulmonary function (FVC, FEV_1, FEV_3, SGaw, FRC) and bronchial reactivity to methacholine were measured following single and sequential exposures. No significant changes in pulmonary function were observed with the exposures. No significant increase in bronchial reactivity occurred with any exposure, although a nearly significant decrease resulted following the single H_2SO_4 exposure. We conclude no decrement in function occurs with exposure to respirable H_2SO_4 aerosol individually or when preceded by 0.3 ppm O_3.

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FOREWORD

The many benefits of our modern, developing, industrial society are accompanied by certain hazards. Careful assessment of the relative risk of existing and new man-made environmental hazards is necessary for the establishment of sound regulatory policy. These regulations serve to enhance the quality of our environment in order to promote the public health and welfare and the productive capacity of our Nation's population.

The Health Effects Research Laboratory, Research Triangle Park, conducts a coordinated environmental health research program in toxicology, epidemiology, and clinical studies using human volunteer subjects. These studies address problems in air pollution, nonionizing radiation, environmental carcinogenesis and the toxicology of pesticides as well as other chemical pollutants. The Laboratory participates in the development and revision of air quality criteria documents on pollutants for which national ambient air quality standards exist or are proposed, provides the data for registration of new pesticides or proposed suspension of those already in use, conducts research on hazardous and toxic materials, and is primarily responsible for providing the health basis for nonionizing radiation standards. Direct support to the regulatory function of the Agency is provided in the form of expert testimony and preparation of affidavits as well as expert advice to the Administrator to assure the adequacy of health care and surveillance of persons having suffered imminent and substantial endangerment of their health.

Research studies involving human volunteer subjects must be conducted under carefully controlled conditions. This report describes a controlled environmental chamber for human exposure to low levels of specific air pollutants, both gases and airborne particulate matter, and the methods for determining the respiratory effects of exposure to these compounds. Ozone and sulfuric acid aerosol are air pollutants associated with transportation and stationary source combustion. This study evaluates changes in pulmonary function and bronchial reactivity as well as subject symptomology with exposure of human subjects to low levels of these pollutants representative of the urban environment or anticipated in possible future pollution episodes.

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Health Effects Research Laboratory

ABSTRACT

PHASE I - SULFURIC ACID AEROSOL. To determine the respiratory effects of low levels of sulfuric acid (H_2SO_4) aerosol, such as those anticipated in ambient air in the near future, 28 normal human subjects were exposed for 4 hours to 98 $\mu g/m^3$, 0.14 μm H_2SO_4 aerosol in an environmentally-controlled exposure chamber. Of the 28 subjects, equal numbers were nonsmokers and cigarette smokers. None of the subjects complained of symptoms attributable to the exposure. Measurements of pulmonary function were obtained 2 hours into the exposure, immediately following exposure, and 2 and 24 hours post-exposure. These measurements were compared with control values obtained at comparable hours on the previous day when the subjects breathed filtered clean air. No significant differences in pulmonary function were observed during the exposure, immediately after exposure, or 2 and 24 hours post-exposure.

PHASE II - OZONE AND SULFURIC ACID AEROSOL. Studies of recent air pollution episodes in the U.S. and Southern England have shown atmospheric coexistence of ozone (O_3) and H_2SO_4 aerosol. Previous exposure of human subjects to low levels of these pollutants (0.3 ppm O_3 and 98 $\mu g/m^3$, 0.14 μm H_2SO_4 aerosol), as separate exposures, produced no significant changes in pulmonary function. Single O_3 exposures to slightly higher levels (0.4-0.5 ppm) have produced decrements in pulmonary function. Repeating this ozone exposure on the following day results in a greater decrement in function occurring on the second day, suggesting an effect of the previous exposure on a subsequent exposure. To determine if an O_3 pre-exposure around "threshold" would produce a significant decrement in function with this H_2SO_4 exposure, 12 healthy nonsmokers were exposed first to 0.3 ppm O_3 for 2 hours, followed by 100 $\mu g/m^3$, 0.13 μm H_2SO_4 aerosol for 4 hours. Separate exposures to O_3 and H_2SO_4 were also done. Three consecutive weeks were employed, one each for O_3, H_2SO_4, and O_3 with H_2SO_4. In each week three consecutive days represented control, exposure, and post-exposure days. Pulmonary function (body plethysmography and spirometry) and bronchial reactivity to methacholine were measured following exposure and 24-hours post-exposure, and compared to control clean-air values. No significant changes in pulmonary function or bronchial reactivity were observed with the ozone, the sulfuric acid aerosol or the sequential ozone-sulfuric acid aerosol exposures. Ozone pre-exposure does not appear to enhance the response to respirable H_2SO_4 aerosol at or near environmentally-observed levels.

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LIST OF ABBREVIATIONS AND SYMBOLS

ABBREVIATIONS

FVC	-- forced vital capacity
FEV_1	-- forced expiratory volume in 1 second
FEV_3	-- forced expiratory volume in 3 seconds
SGaw	-- specific airway conductance
FRC	-- functional residual capacity
RV	-- residual volume
TLC	-- total lung capacity
SBNER	-- single breath nitrogen elimination rate
Phase III	-- alveolar plateau
Phase IV	-- closing volume
C_L stat	-- quasistatic compliance
C_L dyn	-- dynamic compliance
R_L	-- pulmonary resistance
$\dot{V}_E$	-- expired minute ventilation
W	-- watts
Kg-m/min	-- kilogram-meter per minute

SYMBOLS

O_3	-- ozone
H_2SO_4	-- sulfuric acid
NO_x	-- nitrogen oxides
RH	-- hydrocarbons
SO_2	-- sulfur dioxide
SO_3	-- sulfur trioxide
Δ	-- mean of the differences between day 2 and day 1 or between day 3 and day 1 measurements
SE(Δ)	-- standard error of the differences calculated using variance estimate obtained from repeated measures ANOVA (analysis of variance)
Standardized Δ	-- equals Δ /SE(Δ)

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Doctors Robert M. Borushok and Elliott Gorbaty participated in obtaining medical histories, performing physical exams, and assisting in physiological testing. Messrs. Joseph L. Young and Marcus S. Bermel generated, monitored and controlled the ozone gas and respirable sulfuric acid aerosol.

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SECTION 1

INTRODUCTION

This report presents the research results associated with a three-year study funded by the Environmental Protection Agency under Grant Number R803804. The three-year work effort using the University of Maryland Environmental Chamber, consisted of two phases. Phase I investigated the "Effects of Sulfuric Acid Aerosol on Pulmonary Function in Human Subjects". Phase II studied "Pulmonary Function and Bronchial Reactivity in Human Subjects with Exposure to Ozone and Respirable Sulfuric Acid Aerosol".

SULFURIC ACID AEROSOL - PHASES I AND II

Atmospheric sulfur dioxide (SO_2) is oxidized to sulfur trioxide (SO_3) which combines immediately with water vapor to form H_2SO_4 in the form of droplets (1). Cox and Penkett (2) have shown in laboratory experiments that when the photochemical smog reaction, involving unsaturated hydrocarbons (RH) and oxides of nitrogen (NO_x), takes place in the presence of SO_2, oxidation of the SO_2 to H_2SO_4 occurs at a significant rate, even when the concentrations of RH and NO_x were typical of the levels ($\sim$ 0.1 ppm) during moderate pollution episodes.

Sulfuric acid mist has been suggested as one of the irritants contributing to the increased mortality and morbidity resulting from the prolonged polluted fogs of Donora, London, and the Meuse Valley (1,3). Catalytic converters presently installed on automobiles to control pollution may become a source of increased sulfates and sulfuric acid mist in the breathing zone of predestrians and motorists in urban areas (4). Sulfuric acid has been shown to be more toxic than sulfur dioxide in animal and human studies, with the acid particle size and relative humidity affecting toxicity (5,6).

Past measurements (1961) of urban atmospheric levels of sulfuric acid mist in the United States (Los Angeles) show levels up to 50 $\mu g/m^3$ for a 3-hour period (1). With the increased use of high sulfur fuels for combustion and the more widespread use of catalytic-converter equipped automobiles, the peak urban atmospheric burden of H_2SO_4 aerosol of 20 $\mu g/m^3$, estimated by EPA in 1976, could possibly increase to 60-80 $\mu g/m^3$. EPA air quality data reported 24-hour sulfate levels of 162 $\mu g/m^3$ in South Charles, West Virginia in 1973 (7). Air quality data on 2-4 hour sulfate measurements as well as H_2SO_4 aerosol determinations have not been made. Independent researchers have made limited measurements of both the 24-hour sulfate and peak 2-hour sulfate levels. Based on Cass's measurements in the Los Angeles area of peak 2-hour levels being approximately 175% higher than the 24-hour averages (8), the above 24-hour

sulfate level could have 2-hour peak levels of 280 $\mu g/m^3$.

Animal studies have revealed adverse pulmonary function effects to various levels of H_2SO_4 aerosol. Previous studies showed this effect at concentrations $\geq$ 2000 $\mu g/m^3$ (3,9,10,11). Recently, Amdur (12) exposed guinea pigs for one hour to concentrations from 100 to 1000 $\mu g/m^3$ at particle sizes of 0.3 and 1.0 μm. All exposures produced a significant increase in pulmonary flow resistance, which was dose-related. Pulmonary compliance decreased for all exposures, but was not significant for the 100 and 400 $\mu g/m^3$, 1.0 μm exposures.

Amdur et al. (13) reported adverse effects (decreased flow rates) in human subjects exposed by face mask to relatively high concentrations of H_2SO_4 mist (350-500 $\mu g/m^3$ of 1 μm particle size for 5-15 minutes). Sim and Pattle (14) reported symptoms of respiratory tract irritation and increased airway resistance in human subjects exposed to high concentrations of H_2SO_4 acid mist ($\geq$ 3000 $\mu g/m^3$, 1 μm for 10 minutes) in high humidity (90% RH).

More recently, Gardner et al. (15) exposed healthy nonsmoking human subjects for 2 hours to 66, 100, or 195 $\mu g/m^3$, 0.055 μm MMD H_2SO_4 aerosol. The subjects exposed to the lowest concentration seemed to be the most sensitive, with significant decreases in FEV_2 and Raw and an increase in FRC. The group exposed to 100 $\mu g/m^3$ showed significant changes in FEV_2 and Raw, with no significant changes in pulmonary function associated with the subjects exposed to the highest concentration. No significant changes in respiratory rate or tidal volume were observed in this latter group.

Avol et al. (16) reported no effects on pulmonary function in healthy, normal humans and subjects with asthma following exposure to 100 $\mu g/m^3$ H_2SO_4 for 2 hours, and Sackner et al. (17) found no effects following exposure of normals and asthmatics to 100 and 1000 $\mu g/m^3$ for 10 minutes. Utell et al. (18) did find significant changes in flow rates and increased bronchial reactivity, following exposure to 1000 $\mu g/m^3$ H_2SO_4 aerosol for 16 minutes in normal subjects.

The purpose of the Phase I investigation was to determine if exposure of human subjects to levels of H_2SO_4 aerosol anticipated in the near future, in a realistic time frame, would have an adverse effect upon respiratory function. The concentration used was 98 $\mu g/m^3$, with a particle size of 0.14 μm MMD. Exposure duration was 4 hours.

OZONE - PHASE II

Ozone is a photochemical oxidant pollutant formed by the ultraviolet energy of sunlight on the oxides of nitrogen and hydrocarbons primarily emitted from automobiles.

Ozone may represent an occupational hazard for flight crews flying at high altitudes. Young et al. (19) reported estimated concentrations of 0.3 and 0.4 ppm ozone (rubber band cracking method) in the passenger and crew compartments of commercial jet airliners flying between 27,000 and 39,000

feet in altitude. Jaffe and Estes (20) described FAA test studies involving two KC-135 jet aircraft flying at 25,000 to 41,000 feet in altitude; maximum ozone concentration in the aircraft cabin measured 0.5 ppm using a Mast analyzer.

In September 1979 Southern California experienced an episode of photochemical pollution described as "the worst Southern California smog seige in 25 years", with one-hour average concentrations of ozone exceeding 0.4 ppm and eight consecutive days with Stage II ozone ($\geq$ 0.35 ppm) episodes in the South Coast Air Basin (21).

Ozone, the major oxidant in photochemical smog, is a respiratory irritant that has been shown to produce decrements in pulmonary function following low level exposure (0.4-0.5 ppm). Kerr et al. (22) found significant reductions in forced vital capacity (FVC) and specific airway conductance (SGaw), and increased total pulmonary resistance (R_L) following exposure to 0.5 ppm for 6 and 10 hours. Farrell et al. (23) found significant reductions in FVC and SGaw following exposure to 0.4 ppm ozone for 3 hours. Animal studies had previously revealed the phenomenon of tolerance to the respiratory effects of ozone with repeated exposure (24,25,26). More recently this phenomenon of adaptation was demonstrated in human subjects. In a study by Hackney et al. (27), 5 of 6 subjects, exposed to 0.5 ppm for 2 hours per day for 4 consecutive days, showed decreases in pulmonary function on days 1, 2, and 3 that were largely reversed by the fourth day. Farrell et al. (23) demonstrated that when nonsmoking human subjects were exposed to 0.4 ppm ozone for 3 hours per day for 5 consecutive days, decrements in FVC and SGaw occurred on the first two days, which returned to baseline levels by the fifth day despite continued exposure. Folinsbee and co-workers (28) showed that repeated 2-hour exposures to ozone at 0.20 ppm produced no acute effects in pulmonary function; whereas, 2-hour exposures to 0.35 and 0.50 ppm repeated over 3 days produced significant changes in FVC, FEV_1, $FEF_{25-75\%}$. In all these human studies, the maximum decrement in function occurred on the second day of ozone exposure, indicating that there may be some enhancement of sensitivity from the initial ozone exposure. Symptoms generally correlated with decrements in pulmonary function.

Increased bronchial reactivity, as measured by the response to histamine inhalation, has been demonstrated following exposure of normal nonsmoking subjects to 0.6 ppm O_3 for 2 hours (29); this exposure produced no significant changes in baseline airway resistance (Raw), suggesting that bronchial reactivity may be a more sensitive indicator than pulmonary function of changes in the airways following O_3 exposure. The reactivity increase occurred immediately following O_3 exposure, becoming nonsignificant by 24 hours post-exposure. In another study performed in their laboratory, nonatopic and atopic nonsmokers were also exposed to 0.6 ppm O_3 for 2 hours (30). In the nonatopic group, the increase in specific airway resistance (SRaw) produced by either histamine or methacholine aerosol after O_3 exposure was significantly greater than after the sham (air) exposure. For the atopic group inhaling histamine aerosol only, the SRaw increase following O_3 exposure was also significantly greater. In each group, bronchial response was measured one hour after O_3 exposure, the observed increase returning to control 24 hours post-exposure. Baseline SRaw did not change significantly with O_3 exposure.

In both studies subjects were exposed while wearing nose clips, restricting O_3 exposure to oral inhalation only. Although these studies did not show a significant change in pulmonary function (Raw, SRaw), other studies (22,23, 27,28) have demonstrated significant decrements in pulmonary function with exposures to 0.4-0.5 ppm O_3 for 2-3 hours.

SULFURIC ACID AEROSOL WITH OZONE PRE-EXPOSURE - PHASE II

Recent air pollution episode studies performed from 1975 through 1977 in the eastern half of the U.S. from the Great Lakes through the TVA region showed the co-existence of elevated ground-level concentrations of particulate sulfate and ozone as a regional and interstate phenomenon (31). Maximum concentrations were $>$ 0.14 ppm ozone and 40 $\mu g/m^3$ sulfate.

During July 1971 over Southern England significantly higher than normal concentrations of ozone as well as oxidized sulfur dioxide in the form of sulfuric acid and sulfates were measured at ground levels (32). Total sulfate reached levels of 70 $\mu g/m^3$, with the hydrogen ion concentrations expressed as H_2SO_4 aerosol peaking at 55 $\mu g/m^3$. Maximum O_3 levels were 0.10 ppm. A close correspondence in concentration patterns and peaking occurred with the O_3 and H_2SO_4 levels; the H_2SO_4 aerosol also peaked at a lower level earlier in the day when the O_3 concentration was building up.

Previous investigators have studied the combined exposure to ozone and sulfuric acid aerosol, sulfur dioxide or ambient total suspended particulate. Using a mobile laboratory in Los Angeles County during the summer of 1978, Linn et al. (33) demonstrated significant but very small losses in forced expiratory volumes and flows and total lung capacity with very mild clinical symptoms in "allergic" normal and asthmatic subjects exposed to ambient L.A. air of 0.22 ppm ozone and 200 $\mu g/m^3$ total suspended particulate. The H_2SO_4 concentration was not determined. Bedi et al. (34) exposed nine young adult males to 0.40 ppm SO_2, 0.40 ppm O_3, and their combination, each for a 2-hour duration. When exposed to SO_2 alone, no significant changes occurred in pulmonary function; whereas exposure to O_3 or O_3 plus SO_2 produced significant decreases in forced expiratory volumes and maximum expiratory flows. Responses between O_3 alone and O_3 plus SO_2 were not significantly different; thus a synergistic effect was not demonstrated.

Kleinman et al. (35) exposed normal human subjects for 2 hours to 0.37 ppm O_3, 0.37 ppm SO_2, and 100 $\mu g/m^3$, 0.5 μm H_2SO_4 aerosol. The combined exposure showed small statistically significant decrements in forced expiratory function (volumes and flows). The increase in reported symptoms with exposure approached statistical significance. The authors stated that the results did not support the hypothesis that H_2SO_4 aerosol markedly enhances respiratory irritancy of other pollutants (O_3, SO_2); although, a modest degree of enhancement may have occurred compared to previous ozone exposure data.

Last and Cross (36) exposed normal rats to 0.4-0.5 ppm O_3, 1100 $\mu g/m^3$, 0.5 μm H_2SO_4 aerosol, and their combination for 3 and 14 days. Exposure effects were evaluated on conducting airway metabolism as rate of secretion of mucus glycoproteins by tracheal explants and on biochemical parameters in lung homogenates. The authors stated that true synergism was observed in that

the responses to the mixture of O_3 and H_2SO_4 aerosol exceeded the sum of the effects observed with the same concentrations of O_3 and H_2SO_4 presented separately.

Gardner et al. (15) exposed female mice to 900 $\mu g/m^3$, 0.23 μm H_2SO_4 aerosol and 0.1 ppm ozone for 2 and 3 hours respectively, using an infectivity model for evaluating exposure effects. Neither pollutant alone caused a significant increase in mortality with the infectious microorganism challenge as compared to filtered air controls. With sequential exposures to the two pollutants, only when the exposure to the oxidant gas immediately preceded that of the acid (not the acid preceding the ozone) was there a significant increase in respiratory infections, with the observed increased mortality equal to the additive effect of the individual pollutants. A significant decrease in tracheal ciliary activity of hamsters was observed with the same sequential exposure to O_3 followed by H_2SO_4 as compared to the decrease (significant) observed with the H_2SO_4 exposure alone; the O_3 exposure alone resulted in no significant difference from air controls. An additional experiment was conducted using 0.1 ppm O_3 and 500 $\mu g/m^3$ H_2SO_4 aerosol administered simultaneously for a period of 3 hours to female mice, also employing the infectivity model. A significant increase in mortality over air controls was observed, with the acid alone showing no increase and the ozone a nonsignificant increase in mortality.

As noted in the previous section, ozone appears to have the capability of producing a further decrease in pulmonary function with a second exposure. A pre-exposure to this pollutant might have the potential of magnifying the response to respirable sulfuric acid aerosol exposure. Both sulfuric acid aerosol and ozone are pulmonary irritants.

The purpose of the Phase II investigation was to determine if pre-exposure to ozone at a level seen in the urban environment would produce a significant decrement in pulmonary function with an H_2SO_4 aerosol exposure previously shown not to decrease function (37). Concentrations used were 0.3 ppm ozone and 100 $\mu g/m^3$, 0.13 μm sulfuric acid aerosol for 2 and 4 hours respectively. In addition, bronchial reactivity to inhaled methacholine was determined; these measurements appear not to have been made in any published studies.

SECTION 2

CONCLUSIONS

PHASE I - SULFURIC ACID AEROSOL. Single short-term (4 hour) exposure to low levels of respirable sulfuric acid aerosol (98 $\mu g/m^3$ of particle size 0.14 μm) with two 15-minute light-to-moderate exercise periods appears to have no adverse effects on pulmonary function in normal human subjects.

PHASE II - OZONE AND SULFURIC ACID AEROSOL. Single short-term exposure to 0.3 ppm ozone for 2 hours with one 15-minute light-to-moderate exercise period showed no significant changes in pulmonary function or bronchial reactivity to methacholine in normal, nonsmoking human subjects. Exposure to respirable sulfuric acid aerosol (100 $\mu g/m^3$, 0.13 μm H_2SO_4 for 4 hours) with the same light-to-moderate exercise also demonstrated no significant changes in pulmonary function or bronchial reactivity. When the H_2SO_4 aerosol exposure was preceded by the O_3 exposure, no significant changes were seen in pulmonary function or bronchial reactivity. We conclude that there are no readily apparent risks from sequential exposures of nonsmokers to these low levels of ozone and sulfuric acid aerosol at this exercise level.

SECTION 3

RECOMMENDATIONS

PHASE I - SULFURIC ACID AEROSOL. No significant changes in pulmonary function were evident with this study, but longer or repeated exposures may produce function decrements. Exacerbations of chronic bronchitis are known to occur with exposure to high ambient levels of SO_2 and particulates, of which H_2SO_4 aerosol is a constituent.

Further studies should be undertaken to: 1) evaluate the effects of longer or repeated exposures to H_2SO_4 aerosol; 2) evaluate the effects of H_2SO_4 aerosol on subjects with allergy, asthma and/or chronic bronchitis; 3) evaluate the effects of combinations of H_2SO_4 and other constitutents of the SO_2/particulate complex on both normal subjects and subjects with allergy, asthma and/or chronic bronchitis. Investigation of the effects to higher H_2SO_4 concentrations would appear to have little relevance to the effects of air pollution, unless ambient levels increase markedly in the future.

PHASE II - OZONE AND SULFURIC ACID AEROSOL. In normal, nonsmoking human subjects, no significant changes in pulmonary function were observed with individual or sequential exposures to 0.3 ppm O_3 for 2 hours and 100 $\mu g/m^3$, 0.13 μm H_2SO_4 aerosol for 4 hours employing light-to-moderate exercise loads. Bronchial reactivity to methacholine did not show a significant increase with any exposure; although a substantial, nearly-significant decrease occurred following the 4-hour exposure to respirable sulfuric acid aerosol.

Additional studies should be undertaken to: 1) evaluate the effects of ozone and respirable H_2SO_4 aerosol on subjects with allergy, asthma and/or chronic bronchitis; 2) further evaluate the decrease in bronchial reactivity to methacholine following exposure to respirable H_2SO_4 aerosol, i.e. expose additional subjects to 4 hours of 100 $\mu g/m^3$, 0.1-0.3 μm H_2SO_4 aerosol; 3) evaluate the effects of these two air pollutants employing moderate-to-heavy exercise loads.

SECTION 4

MATERIALS AND METHODS

ENVIRONMENTAL CHAMBER

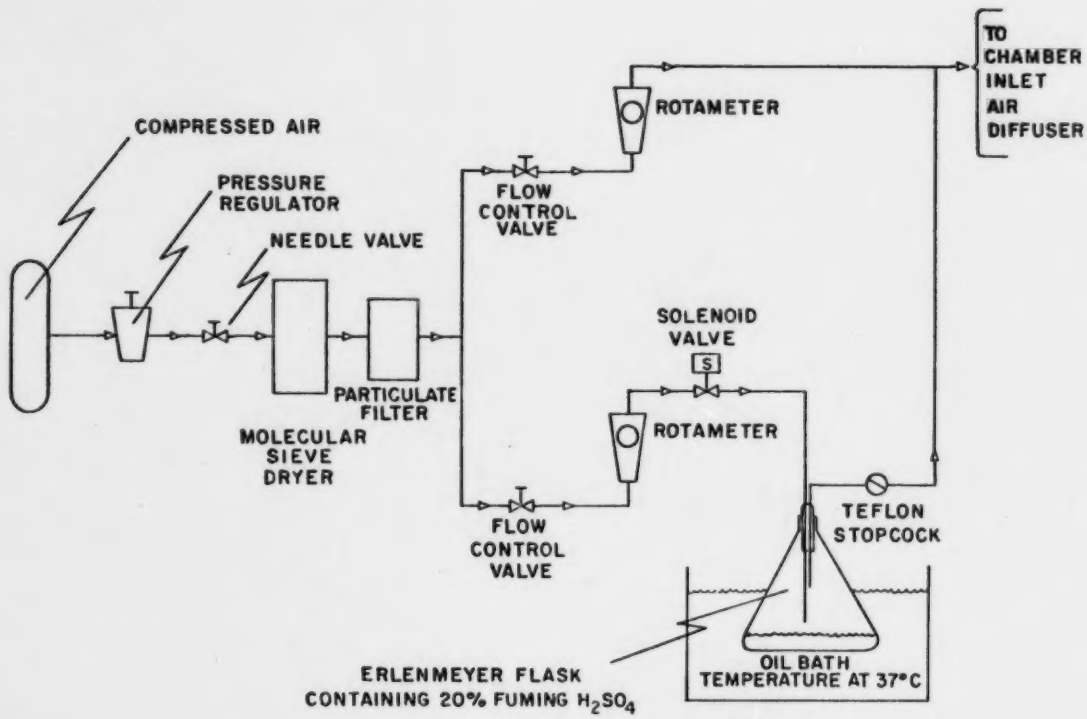
The Phase I and Phase II research studies were conducted in an environmentally controlled chamber at the University of Maryland, restricting environmental air pollutants to the generated ozone or sulfuric acid aerosol. The chamber was designed so that air entering passed through high efficiency particulate absolute (HEPA) filters and activated carbon filters, permitting cleanliness approaching class 100, ($\prec$ 100 particles $\geqslant 0.5$ μm per foot^3 or $\prec$ 3534 particles $\geqslant 0.5$ $\mu m/m^3$). All air was exhausted to the outside, and no recirculation was permitted. The ventilation rate of 8.49 m^3/min. (300 ft.^{3}/min.) in the 22.2 m^3 (784 ft.^{3}) chamber enabled a complete air change every 2.6 minutes. Temperature was maintained at 22.2 $\pm$ 0.5°C (72 $\pm$ 1°F) and relative humidity at 60 $\pm$ 2 per cent. A more detailed description of the environmental chamber was presented in a previous publication (38).

PHASE I STUDY - EFFECTS OF SULFURIC ACID AEROSOL ON PULMONARY FUNCTION IN HUMAN SUBJECTS.

Aerosol Generation and Monitoring

The aerosol was generated by the reaction of SO_3 gas with water vapor similar to the method described by Scaringelli and Rehme (39). Compressed cylinder air was dried by a molecular sieve column and passed through a glass fiber filter to take out particulate matter. The air was then split, one airstream passing through a temperature-controlled Erlenmeyer flask containing 250 ml of approximately 20% fuming sulfuric acid. This SO_3 laden air was diluted by the second air stream and brought to the room's air inlet diffuser where rapid mixing occurred. The SO_3 reacted with the room's water vapor immediately to form a submicronic aerosol. Stainless steel tubing (0.4 cm. I.D., 0.6 cm. O.D.) and Stainless Steel Swagelok^R fittings were used throughout the system. Heating tapes placed around the 15 feet of stainless steel tubing from the generator to the room air inlet diffuser, maintained the air temperature inside the tubing at 160 $\pm$ 10°C. Maintaining this temperature prevented the formation of H_2SO_4 droplets on the inner surface of the tubing. The mass concentration of the acid in the room was adjusted by flow control valves and rotameters; flow through the system was approximately 500 c.c./min. Refer to Figure 1.

Aerosol samples were collected onto 47 mm diameter Fluoropore^R filters having 1 μm pore size (Millipore Corp., Bedford, Mass.) held in a Millipore



SCHEMATIC DIAGRAM OF THE AEROSOL GENERATION SYSTEM

stainless steel filter holder. Each filter was flow calibrated before use. The volume of air sampled was at least 1 m^3 and the amount of aerosol collected was 100 μg or more. Sampling was done every hour. After sampling, the filter was placed in a beaker containing 30 ml of distilled water, face down. The beaker was covered with Parafilm M^R (American Can Co., Greenwich, CT) and sonified for eight minutes in an ultrasonic cleaner (Mettler Electronic Corp., Anaheim, CA) to extract the collected acid. After extraction, the sample was analyzed twice in 10 ml alequots for total soluble sulfate by titrating with 0.005 M barium perchlorate in an 80% isopropanol solution, using thorin as the end-point indicator (40). The average of the two determinations was taken. Knowing the amount of sulfate (SO_4^{--}) collected and the volume of air sampled, the mass concentration of the acid in the room can be determined. Reference filter strips supplied by the EPA Quality Assurance Laboratory were analyzed using this method and good correlation was noted with EPA data.

Instrumental Methods - Sulfuric Acid Aerosol

For each instrument, the signal output is an indication of the amount of aerosol present. However, because of difference in design, method of detection, and sensitivity to particle size and shape, the instruments were calibrated by comparing the instrument read-outs with results from the wet chemical analysis of filter samples collected concurrently.

Meloy SA-285 Total Sulfur Analyzer (Meloy Labs Inc., Springfield, VA)

The Meloy Analyser was a modified unit in which the aerosol went directly from the inlet port to the Flame Photometric Detection (FPD) burner block, by-passing the solenoid valves. This modification permitted determination of the aerosol concentration with minimal particle size dependence. The unit was factory calibrated using sulfur dioxide gas. This unit was used to give information on the consistency of the mass concentration of the sulfuric acid aerosol in the environmental chamber. Calibration was by wet chemical sulfate analysis of filter samples.

Electrical Aerosol Analyser (EAA - Thermo-Systems Inc., St. Paul, MN)

The EAA was used to provide data on particle size distribution and mass concentration of the H_2SO_4 aerosol. The instrument detects particles in the size range from 0.0042 through 0.75 μm in diameter. Knowing the volume distribution and the particle density, a mass distribution can also be obtained. In the environmental chamber, where the relative humidity was maintained at 60% and temperature at 22.2°C, the particle density was 1.3 g/cm^3 or 0.38 g H_2SO_4/g solution.

The H_2SO_4 aerosol in the chamber had a mass median diameter (MMD) of 0.14 μm and a geometric standard deviation (σg) about 2.9. The variability of the aerosol mass concentration was 98 $\pm$ 9 ug/m^3.

Subjects

The 28 subjects (14 smokers and 14 nonsmokers) selected for the investigation had no history of chronic respiratory or cardiovascular disease, and had normal findings on physical examination. They ranged in age from 18 to 45 years with a mean age of 24 years (Table 1). There were 19 males and 9 females, of which the nonsmokers included 8 males and 6 females. The subjects were studied singly or in pairs, and each served as his or her own control. The smokers were asked to refrain from smoking on the morning of the study, and were not permitted to smoke during the study.

Chamber Procedure

The studies were carried out on three consecutive days, at the same time each day. During the first and third days the subjects breathed only filtered clean air for a six hour period; on the second day, they breathed sulfuric acid aerosol at a concentration of 98 $\mu g/m^3$ for 4 hours, and filtered clean air for an additional 2 hours. The second day was always the exposure day but the subjects did not know on which day they would be exposed. This modified double blind exposure was possible because of the absence of taste or odor at this concentration of H_2SO_4.

On each day pulmonary function tests, FVC, FEV_1, FEV_3, FRC, Raw and SBNER were performed immediately prior to exposure, and at 2-hour intervals thereafter. At 4 hours, quasistatic and dynamic compliance and pulmonary resistance were also measured.

At one and three hours into the study on each day, bicycle ergometer exercise was performed at a work load of 100 watts at 60 RPM for 15 minutes. The exercise was performed to more closely simulate normal activity of urban dwellers, who would be expected to engage in some exercise during the course of the day.

Physiological Tests

Pulmonary function studies were timed so that measurements were made prior to exposure, two hours into exposure, immediately post-exposure, and 2 and 24 hours post-exposure. These measurements were compared with the control day during which the subjects breathed only filtered clean air. Pulmonary resistance and compliance measurements were made only once a day to minimize subject discomfort. To ensure technically satisfactory subject performance, the subjects repeatedly executed each test in practice sessions before the study, except those involving the esophageal balloon.

The forced vital capacity (FVC) was performed using a waterseal direct-writing spirometer. All volumes were expressed in liters adjusted to body temperature, pressure, saturated (BTPS).

Airway resistance (Raw) and volume of thoracic gas (VTG) were measured by the whole body pressure plethysmographic technique of DuBois and associates (41,42) with some modifications (38). The Raw was expressed in units of specific airway conductance (SGaw=1/Raw/Vtg). The SGaw was expressed as

TABLE 1

ANTHROPOMETRIC DATA - PHASE I STUDY

	SMOKERS	NON-SMOKERS
Mean Age (year)	24	24
Age Range (year)	19-38	18-45
Mean Height (cm)	175	175
Height Range (cm)	152-185	160-183
Male	11	8
Female	3	6

1/(cm H_2O x sec) units.

Tests of N_2 elimination rate (SBNER) were calculated from an XY plot of expired N_2 concentration versus expired volume from TLC to RV, after a full inspiration of 100 per cent O_2 from RV to TLC. The rate of expiration was controlled so as not to exceed 0.5 liter/sec. Phase III was expressed as change in per cent of alveolar N_2 concentration per liter of expired volume, and was determined from the best fit straight line of the alveolar sample midportion of the XY plot. Phase IV (closing volume) was measured from RV to the phase III junction with phase IV (43). One reader performed all of the line-fitting procedures.

Pulmonary resistance (R_L) and compliance (C_L) were determined by the electronic subtractor method of Mead and Whittenberger (44). Dynamic compliance was performed at frequencies of 16 breaths per min ($D_L$dyn 16), 32 breaths per min, and 48 breaths per min in rhythm with an audio metronome while maintaining a tidal volume of 1 liter for 8 to 10 breaths at each breathing frequency. To stabilize volume history, each subject fully inflated his or her lungs to TLC before performing the quasistatic and dynamic compliance maneuvers. To determine $C_L$dyn, a portion of the transpulmonary pressure proportional to airflow was substracted by manual potentiometer adjustment for the best-fit straight line XY oscilloscope display of this resultant pressure versus tidal volume. For R_L, a portion of transpulmonary pressure proportional to volume change was subtracted in a similar fashion, with straight line fitting of the XY plot of this pressure versus airflow. All subtractions and readings were performed on playback of analog tape recordings of airflow, volume change, and transpulmonary pressure. This procedure enabled rereading of the recordings to verify values and also to standardize the length of time each subject performed at the various breathing frequencies, thereby avoiding excessive alveolar ventilation from prolonged measurement. Pulmonary resistance was expressed as cm H_2O/liter/sec., and was not corrected for the Vtg at which it was measured. All compliance data were expressed as liters/cm H_2O.

Data Analysis

Quasistatic compliance, dynamic compliance and total pulmonary resistance for the group of 28 subjects were examined by the Student t-test for paired observations. The post-exposure measurements were each compared with control values. This type of analysis was suitable because these measurements were performed only once each day.

The remaining physiological data were examined by analysis of variance for factorial design. This statistical parameter was employed because it was necessary to consider day - hour interaction. These data were also subjected to orthogonal polynomial analysis to determine if there were any significant trends.

PHASE II STUDY - PULMONARY FUNCTION AND BRONCHIAL REACTIVITY IN HUMAN SUBJECTS WITH EXPOSURE TO OZONE AND RESPIRABLE SULFURIC ACID AEROSOL.

Ozone Generation and Monitoring

Ozone was produced by passing 100 per cent O_2 through a water-cooled, silent-arc, ultraviolet ozone generator, and was introduced via stainless steel tubing into the exposure room of the chamber through the room air input diffuser. The ozone concentration in the chamber was continuously monitored by a microcoulomb ozone sensor (Mast Model 724-5 Oxidant Monitor) and a chemiluminescent monitor (Monitor Labs Model 8410 Ozone Analyzer). The 2 monitors were calibrated at the National Bureau of Standards using the Federal Register neutral-buffered potassium iodide method. Mean readings from each monitor were in agreement to within 0.01 ppm. In addition, calibration of the monitors was performed before and after the exposure studies using a Monitor Labs Model 8500 Calibrator with internal ozone generator. Ozone monitor strip chart recordings were made during exposures. The mean of the various 2-hour exposures was 0.30 ppm ozone, with measurements ranging between 0.30 and 0.31 ppm.

Sulfuric Acid Aerosol Generation and Monitoring

The aerosol was generated by the reaction of SO_3 gas obtained from fuming sulfuric acid with the water vapor in the chamber exposure room, as described in the Phase I Study portion of Section 4 on page 8. Aerosol samples were collected during exposure in the chamber room and analyzed by wet chemical analysis of the filter samples as described on page 8. Continuous monitoring of the room aerosol concentration and particle size distribution was also as discussed earlier (page 10). The H_2SO_4 aerosol in the chamber had a mass median diameter (MMD) of 0.13 μm and a geometric standard deviation (σg) about 2.4. The variability of the aerosol mass concentration was 100 $\pm$ 8 $\mu g/m^3$.

Subjects

The 12 subjects, all nonsmokers, selected for the investigation had no history of chronic respiratory or cardiovascular disease and had normal findings on physical examination. They ranged in age from 19 to 46 years with a mean age of 25 years (Table 2). There were 7 males and 5 females. The subjects were studied singly or in pairs, and each served as his or her own control. Informed consent was obtained from each subject after the nature of the procedure had been fully explained and preliminary pulmonary function tests, a medical history, and physical exam had been successfully completed.

Chamber Procedure

A three-week study procedure was employed. The subjects were confined to the exposure room of the environmental chamber on three consecutive days of three consecutive weeks as follows:

TABLE 2. ANTHROPOMETRIC DATA AND SYMPTOMS DURING EXPOSURE - PHASE II STUDY

Subject	Sex	Height (cm)	Age (years)	Weight (kg)	Cough*	Chest Discomfort*	Throat Irritation*	Headache or Lightheadness*
1	M	180	46	75	O3	X	X	X
2	M	178	19	66	O1,O3	O1,O3,S3	X	X
3	M	185	22	91	O3	O3	X	X
4	M	180	22	65	O1	O3	X	X
5	F	170	21	50	X	X	S3	X
6	F	170	24	75	X	X	O1,S2	O3
7	F	169	22	73	X	X	X	X
8	M	175	25	68	X	X	S3	O1
9	M	178	22	72	X	X	X	X
10	F	160	27	59	X	X	X	X
11	F	168	22	89	X	O3	X	X
12	M	173	28	77	X	X	X	X
Mean		174	25	72				
Range		160-185	19-46	50-91				

* X, no symptoms experienced
O1, symptom experienced during ozone exposure of First Week
S2, symptom experienced during sulfuric acid aerosol exposure of Second Week
O3,S3, symptom experienced during ozone pre-exposure (O3) followed by sulfuric acid aerosol exposure (S3) of Third Week

Procedure Routine	1st Day Control	2nd Day Exposure	3rd Day Post-exposure
1st Week (2 hours/day)	Air only	0.3 ppm O_3	Air only
2nd Week (4 hours/day)	Air only	100 $\mu g/m^3$ H_2SO_4	Air only
3rd Week (6 hours/day)	Air only	O_3, then H_2SO_4	Air only

For each week, the study was conducted at the same time period of each of the three days to avoid diurnal variation in pulmonary function (38). During the first and third days, the subjects breathed only filtered clean air for 2, 4, and 6 hours on the first, second, and third weeks respectively. On the second day, they breathed filtered air plus 0.3 ppm O_3 for 2 hours on the first week, 100 $\mu g/m^3$, 0.13 μm H_2SO_4 aerosol for 4 hours on the second week, and 0.3 ppm O_3 for 2 hours followed by 100 $\mu g/m^3$, 0.13 μm H_2SO_4 aerosol for 4 hours on the third week.

Each day one hour prior to exiting from the chamber exposure room, bicycle ergometer exercise was performed at a work load at 100 watts (612 Kilogram-meters/minute) at 60 RPM for 15 minutes. The exercise was performed to ensure a short period of increased ventilation and to more closely simulate the normal activity of urban dwellers, who would be expected to engage in some exercise during the course of the day. Pulmonary function and methacholine challenge tests were performed by each subject in the environmental chamber clean room immediately following exit from the chamber exposure room.

Physiological Tests

Pulmonary function tests (PFTs) were performed in sequence (plethysmography, then spirometry) following the 2, 4, or 6-hour confinement in the chamber exposure room each day of the 3-week study. Bronchial reactivity to methacholine was measured immediately following the spirometry tests. These measurements were taken on Day 1 (Control), Day 2 (Exposure), and Day 3 (Post-exposure) of each week; the exposure day and post-exposure day measurements were compared with those of the control day. All PFTs were performed in a seated position to ensure comparability of lung volumes from the various tests. To assure technically satisfactory subject performance, the subjects repeatedly executed each PFT during preliminary practice sessions.

Ventilatory function testing was performed as a single forced vital capacity (FVC) by standard technique employing a waterseal direct-writing spirometer. All volumes were expressed in liters at body temperature, pressure, saturated (BTPS).

Airway resistance (Raw) and volume of thoracic gas (Vtg) were determined by the whole-body pressure plethysmographic technique of DuBois et al. (41,42) Airway resistance measurements determined at a 1 liter/sec airflow (0.5 liter/sec inspiratory to 0.5 liter/sec expiratory) were converted to specific airway conductance (SGaw = 1/Raw/Vtg), using the Vtg at which each Raw was measured. Practice sessions enabled most subjects to perform the panting

maneuvers of Raw and Vtg at or very close to FRC. SGaw was expressed as $1/(cm\ H_2O \times sec)$ units.

Total lung capacity (TLC) was determined as the sum of FRC and the inspiratory capacity, and residual volume (RV) was determined as the difference between FRC and the expiratory reserve volume. All lung volumes were expressed in liters.

The methacholine challenge test was performed by the method of Chai et al. (45) with some modifications. Methacholine aerosol was administered in successively increasing doses and delivered by inhalation through a DeVilbiss No. 646 nebulizer. The nebulizer was attached to a dosimeter and a compressed air source. A breath-activated thermister triggered the dosimeter to supply the nebulizer with compressed air at 20 psi for 0.6 sec., providing the subject with a controlled dose. The nebulizer containing the solution of methacholine was weighed before and after each subject's inhalations to determine the precise amount delivered. The nine concentrations of methacholine varied from 0.078 mg/ml (M1) to 25 mg/ml (M9). Each subject received five inhalations of each concentration until a 40% reduction in SGaw occurred. The logarithm dose of methacholine which decreased the SGaw by 35% from control, called the log provocative dose (log PD_{35}), was then derived mathematically by linear regression analysis. Aerosolized Bronkosol^R was available to treat bronchospasm should it be needed. A decrease in the log PD_{35} determination represents an increase in bronchial reactivity.

Data Analysis

The physiologic data for the group of 12 subjects were examined by Tukey's Test. The exposure (Day 2) measurements for the pulmonary function tests and the methacholine challenge tests were each compared with the control (Day 1) values, and the post-exposure (Day 3) measurements also were each compared with the control (Day 1) values. The resulting differences were standardized by dividing each by its standard error. The statistical significance of these standardized differences was determined by the Tukey's Test (46,47). The variance estimates used in the computation were obtained from a repeated-measures analysis of variance. A level of $p \leq 0.05$ was used to establish statistical significance.

SECTION 5

EXPERIMENTAL PHASE (RESULTS)

PHASE I STUDY - EFFECTS OF SULFURIC ACID AEROSOL ON PULMONARY FUNCTION IN HUMAN SUBJECTS.

None of the subjects complained of symptoms attributable to exposure; subject bias was removed because the subjects did not know on which of the 3 days they were exposed.

No significant changes were found in any parameter of pulmonary function as a result of the exposure, either during the exposure, immediately following, or 2 and 24 hours post-exposure (Tables 3 and 4).

Data for smokers and nonsmokers were analyzed separately. As might be expected, significant differences were found between the two groups with the single breath nitrogen elimination data (the slope of phase III); however, there were no significant day or hour interactions, indicating no difference in the response to H_2SO_4 aerosol exposure between smokers and nonsmokers. Orthogonal polynominal data analysis, both linear and quadratic revealed no significant trends.

PHASE II STUDY - PULMONARY FUNCTION AND BRONCHIAL REACTIVITY IN HUMAN SUBJECTS WITH EXPOSURE TO OZONE AND RESPIRABLE SULFURIC ACID AEROSOL.

Subjects generally were unable to sense the presence of sulfuric acid aerosol because of the absence of taste or odor at the 100 $\mu g/m^3$ concentration; although, 3 of 12 subjects did experience throat irritation with the exposure. However, the odor of ozone was readily perceptible at the 0.30 ppm concentration; thus, the impracticality of conducting a double-blind study.

Subjects were asked to keep note of the symptoms they experienced. Following each day's confinement in the chamber, they were asked about symptoms, especially cough, chest discomfort, and irritation of the mucous membranes. Symptoms predominated with the ozone exposures of the first and third weeks (Table 2). Of the 12 subjects, one experienced mild symptoms with the H_2SO_4 exposure (Week 2) and three experienced mild symptoms with the H_2SO_4 exposure following the O_3 exposure (Week 3). Four subjects had no symptoms throughout the 3-week study.

No significant changes in pulmonary function were associated with the 2-hour ozone exposure in the first week (Table 5). Bronchial reactivity to methacholine showed a small nonsignificant increase following the ozone

TABLE 3

MEAN PULMONARY FUNCTION MEASUREMENTS - PHASE I STUDY

Test	Hour	Day 1 (Control)	Day 2 (Exposure)	Day 3 (Post-exposure)	ANOVA	F	DF
FVC	0	5.12	5.06	5.00	Day	0.212	2/312
	2	5.08	5.02	4.98	Hour	0.036	3/312
	4	5.05	5.01	5.00	Interaction	0.007	6/312
	6	5.05	5.00	4.97			
FEV_1	0	4.23	4.22	4.15	Day	0.007	2/312
	2	4.20	4.14	4.15	Hour	0.023	3/312
	4	4.15	4.14	4.23	Interaction	0.061	6/312
	6	4.17	4.19	4.19			
FEV_3	0	4.98	4.93	4.88	Day	0.128	2/312
	2	4.93	4.86	4.85	Hour	0.065	3/312
	4	4.90	4.87	4.88	Interaction	0.010	6/312
	6	4.90	4.85	4.83			
FRC	0	3.20	3.19	3.12	Day	0.711	2/312
	2	3.18	3.03	3.01	Hour	1.058	3/312
	4	3.11	3.04	3.04	Interaction	0.114	6/312
	6	3.05	2.95	3.01			
SGaw	0	.184	.175	.178	Day	0.173	2/312
	2	.185	.176	.184	Hour	0.256	3/312
	4	.183	.188	.187	Interaction	0.154	6/312
	6	.183	.182	.183			
SBNER	0	1.26	1.19	1.12	Day	0.387	2/312
	2	1.10	1.09	1.09	Hour	1.513	3/312
	4	1.09	1.10	1.06	Interaction	0.099	6/312
	6	1.06	1.04	1.02			
Phase IV	0	.36	.36	.40	Day	0.391	2/312
	2	.39	.42	.42	Hour	0.453	3/312
	4	.42	.39	.45	Interaction	0.523	6/312
	6	.41	.41	.43			

TABLE 4

COMPLIANCE AND PULMONARY RESISTANCE - PHASE I STUDY

Test	Day 1 (Control)	Day 2 (Exposure)	Day 3 (Post-exposure)	Student's t-test Day 2	Day 3
C_L stat	0.25	0.25	0.26	t = 0.72	t = 0.86
C_L dyn 48	0.25	0.24	0.26	t = 1.36	t - 0.86
R_L	2.50	2.50	2.50	t = 0.09	t = 0.22

TABLE 5. MEAN PULMONARY FUNCTION MEASUREMENTS WITH OZONE EXPOSURE - PHASE II STUDY

Test	Day 1 (Control)	Day 2 (Exposure)	Day 3 (Post-exposure)	Tukey's Test: Day 2 - Day 1 Mean of Differences (Δ) $\pm$ SE (Δ)	Day 2 - Day 1 Standardized Delta (Δ)*	Day 3 - Day 1 Mean of Differences (Δ) $\pm$ SE (Δ)	Day 3 - Day 1 Standardized Delta (Δ)*
FVC	4.84	4.74	4.74	-.09 $\pm$ .053	-1.70	-.09 $\pm$ .053	-1.70
FEV_1	4.00	3.93	4.06	-.07 $\pm$ .058	-1.20	.06 $\pm$ .058	1.03
FEV_3	4.68	4.62	4.63	-.06 $\pm$ .055	-1.09	-.05 $\pm$ .055	-0.90
SGaw	0.172	0.166	0.179	-.006 $\pm$ .0058	-1.04	.007 $\pm$ .0058	1.21
FRC	2.89	2.94	2.99	.05 $\pm$ .048	1.04	.09 $\pm$ .048	1.88
TLC	6.23	6.20	6.22	-.03 $\pm$ .080	-0.37	-.02 $\pm$ .080	-0.25
RV	1.40	1.46	1.47	.07 $\pm$ .064	1.10	.08 $\pm$ .064	1.26

* Standardized $\Delta = \frac{\Delta}{SE(\Delta)}$, $SE(\Delta)$ calculated using variance estimate obtained from repeated measures ANOVA.

exposure (Table 8). The 4-hour respirable H_2SO_4 aerosol exposure of the second week did not produce any significant changes in pulmonary function (Table 6). Bronchial reacivity with the aerosol exposure (Table 8), however, showed a substantial decrease approaching significance (2.22 vs 2.53 for $p = 0.05$). This represents a near-significant decrease in the subject's bronchial airway sensitivity to methacholine.

The third week of the study involved exposing the subjects again to the 0.3 ppm O_3 for two hours followed by the 100 $\mu g/m^3$, 0.14 μm H_2SO_4 aerosol exposure for four hours. Exposure of these subjects to the same concentration of ozone as received during the first week again resulted in no significant changes in pulmonary function (Table 7). Exposing the subjects to H_2SO_4 aerosol following the O_3 exposure did not produce a significant change in pulmonary function (Table 7) or bronchial reactivity to methacholine (Table 8). Although no significant changes in bronchial reactivity were observed with any exposure, a substantial nearly-significant <u>decrease</u> in reactivity occurred with the individual H_2SO_4 aerosol exposure and a smaller, nonsignificant <u>increase</u> in reactivity resulted with the individual O_3 exposure. With the sequential O_3-H_2SO_4 exposure, a negligible change in reactivity was observed.

In summary, no significant changes in pulmonary function or bronchial reactivity were associated with any exposure. The only strong trend toward significance was seen as a decrease in bronchial reactivity associated with the H_2SO_4 aerosol exposure. The bronchial reactivity data of one of the 12 subjects had to be dropped from the statistical analysis, because of a technical error associated with the challenge tests for that subject.

TABLE 6. MEAN PULMONARY FUNCTION MEASUREMENTS WITH SULFURIC ACID AEROSOL EXPOSURE - PHASE II STUDY

Test	Day 1 (Control)	Day 2 (Exposure)	Day 3 (Post-exposure)	Tukey's Test: Day 2 - Day 1 Mean of Differences (Δ) $\pm$ SE (Δ)	Tukey's Test: Day 2 - Day 1 Standardized Delta (Δ)*	Tukey's Test: Day 3 - Day 1 Mean of Differences (Δ) $\pm$ SE (Δ)	Tukey's Test: Day 3 - Day 1 Standardized Delta (Δ)*
FVC	4.85	4.77	4.76	-.07 $\pm$ .131	-0.53	-.09 $\pm$ .131	-0.69
FEV_1	4.02	4.03	4.08	.01 $\pm$ .107	0.09	.07 $\pm$ .107	0.66
FEV_3	4.66	4.65	4.66	-.01 $\pm$ .114	-0.09	.00 $\pm$ .114	0.00
SGaw	0.185	0.189	0.187	.004 $\pm$ .0066	0.61	.002 $\pm$ .0066	0.30
FRC	2.92	2.92	2.94	.01 $\pm$ .050	0.20	.02 $\pm$ .050	0.40
TLC	6.27	6.15	6.22	-.12 $\pm$ .145	-0.83	-.05 $\pm$ .145	-0.34
RV	1.43	1.38	1.46	-.05 $\pm$ .090	-0.55	.03 $\pm$ .090	0.33

* Standardized $\Delta = \frac{\Delta}{SE(\Delta)}$, $SE(\Delta)$ calculated using variance estimate obtained from repeated measures ANOVA.

TABLE 7. MEAN PULMONARY FUNCTION MEASUREMENTS WITH OZONE PRE-EXPOSURE FOLLOWED BY SULFURIC ACID AEROSOL EXPOSURE - PHASE II STUDY

Test	Day 1 (Control)	Day 2 (Exposure)	Day 3 (Post-exposure)	Tukey's Test: Day 2 - Day 1 Mean of Differences (Δ) ± SE (Δ)	Tukey's Test: Day 2 - Day 1 Standardized Delta (Δ)*	Tukey's Test: Day 3 - Day 1 Mean of Differences (Δ) ± SE (Δ)	Tukey's Test: Day 3 - Day 1 Standardized Delta (Δ)*
FVC							
O_3	4.71	4.66	----	-.05 ± .048	-1.04	----	----
H_2SO_4	4.75	4.70	4.76	-.04 ± .048	-0.84	.01 ± .048	0.21
FEV_1							
O_3	4.02	3.97	----	-.05 ± .051	-0.98	----	----
H_2SO_4	4.03	4.01	4.06	-.01 ± .051	-0.19	.04 ± .051	0.78
FEV_3							
O_3	4.59	4.55	----	-.05 ± .048	-1.03	----	----
H_2SO_4	4.64	4.59	4.63	-.06 ± .048	-1.24	-.01 ± .048	-0.21
SGaw							
O_3	0.197	0.185	----	-.012 ± .0075	-1.59	----	----
H_2SO_4	0.193	0.199	0.203	.006 ± .0075	0.80	.010 ± .0075	1.33
FRC							
O_3	2.88	2.92	----	.04 ± .029	1.38	----	----
H_2SO_4	2.92	2.94	2.94	.02 ± .029	0.69	.03 ± .029	1.03
TLC							
O_3	6.17	6.16	----	-.01 ± .097	-0.10	----	----
H_2SO_4	6.22	6.18	6.21	-.04 ± .097	-0.41	-.01 ± .097	-0.10
RV							
O_3	1.48	1.50	----	.02 ± .097	0.21	----	----
H_2SO_4	1.47	1.48	1.45	.01 ± .097	0.10	-.02 ± .097	-0.21

* Standardized $\Delta = \frac{\Delta}{SE\ (\Delta)}$, SE(Δ) calculated using variance estimate obtained from repeated measures ANOVA.

TABLE 8. BRONCHIAL REACTIVITY TO METHACHOLINE - PHASE II STUDY

	Mean Log Provocative Dose (Log PD_{35})			Tukey's Test			
				Day 2 - Day 1		Day 3 - Day 1	
	Day 1 (Control)	Day 2 (Exposure)	Day 3 (Post-exposure)	Mean of Differences (Δ) $\pm$ SE (Δ)	Standardized Delta (Δ)*	Mean of Differences (Δ) $\pm$ SE (Δ)	Standardized Delta (Δ)*
Ozone	1.69	1.57	1.71	-.11 $\pm$ .105	-1.05	.02 $\pm$ .105	0.19
Sulfuric Acid Aerosol	1.39	1.65	1.59	.26 $\pm$ .117	2.22	.20 $\pm$ .117	1.71
Ozone Pre-exposure Followed by Exposure to Sulfuric Acid Aerosol	1.34	1.34	1.39	.03 $\pm$ .077	0.39	.08 $\pm$ .077	1.04

* Standardized$\Delta = \frac{\Delta}{SE(\Delta)}$, $SE(\Delta)$ calculated using variance estimate obtained from repeated measures ANOVA.

SECTION 6

DISCUSSION

PHASE I STUDY - EFFECTS OF SULFURIC ACID AEROSOL ON PULMONARY FUNCTION IN HUMAN SUBJECTS.

It has been suggested that the H_2SO_4 component of air pollution may have been responsible for the increased mortality and morbidity resulting from the prolonged fogs of London, Donora and the Meuse Valley (1,3). This study shows that no adverse effects on pulmonary function are attributable to exposure to respirable 98 $\mu g/m^3$ H_2SO_4 aerosol for 4 hours. Recent studies by Avol (16) and Sackner (17) showed no decrements in pulmonary function with exposure to the same concentration for 10 minutes and 2 hours, and to 1000 $\mu g/m^3$ for 10 minutes. Utell et al. (18) found significant changes in MEFR and PEFR at 60% and 40% of TLC, and increased airway reactivity following exposure to 1000 $\mu g/m^3$ for 16 minutes. The present study provides a larger sample for analysis, a longer period of exposure (4 hours), and data from smokers and nonsmokers. The four-hour exposure period was justified because ambient air sulfate measurements, made at 2-hour intervals at monitoring stations in the Los Angeles area during July 1977, showed a diurnal variation in total sulfate concentration, with peak values for 2 to 4 hours as high as 40-60 $\mu g/m^3$ (Personal Communication - G.R. Cass, Calif. Inst. Tech.). Measurements of H_2SO_4 aerosol were not made; this aerosol constitutes a majority of the ambient acid sulfate. Although H_2SO_4 aerosol represents a small portion of the total sulfate observed, it is expected that the H_2SO_4 aerosol would also have a similar diurnal pattern.

The level of H_2SO_4 aerosol used in this study is higher than present U.S. ambient levels but assumed to be lower than the levels recorded during the great London Fog of 1962. This level was chosen as representative of a moderate increase in H_2SO_4 concentration that might be expected with the increased use of automobiles equipped with catalytic converters, and the increased use of high sulfur fuels, i.e. coal, that may be necessary because of the anticipated shortage of oil.

The fact that no significant effects were noted with this exposure is of interest in view of the suspicion that H_2SO_4 was the component responsible for the increased mortality associated with 'sulfur oxide-particulate' pollution (1). Studies with ozone reveal decrements in pulmonary function with low level exposure (23,28), but epidemiological studies have failed to show increased mortality as a result of elevated ambient ozone levels (48). Sim and Pattle have shown an accumulative effect from H_2SO_4 exposure (14) which suggests that, although no significant changes were noted with our 4-hour exposure, repeated exposures over several successive days may be harmful. Repeated exposures to ozone, on the other hand, result in adaptation (23) which may explain the

lack of conclusive evidence for ozone associated mortality in those with chronic respiratory disease. High ambient levels of H_2SO_4 aerosol are invariably associated with high levels of other pollutants particularly SO_2, carbon aerosol and $(NH_4)_2SO_4$. Synergism between these individual pollutants may play a role in the observed increased morbidity and mortality.

PHASE II STUDY - PULMONARY FUNCTION AND BRONCHIAL REACTIVITY IN HUMAN SUBJECTS WITH EXPOSURE TO OZONE AND RESPIRABLE SULFURIC ACID AEROSOL.

Ozone and sulfuric acid aerosol are two air pollutants resulting from automobile exhaust emissions. Of concern is their possible synergistic health effects, since recent air pollution episode studies in the eastern U.S. demonstrated the co-existence of elevated ground-level concentrations of particulate sulfate and ozone (31). Sulfuric acid aerosol may have contributed to the increased mortality and morbidity resulting from the prolonged fogs of London, Donora, and the Meuse Valley (1,3). Increased use of high sulfur containing fuels for combustion and more widespread use of catalytic-converter equipped automobiles could increase the atmospheric level of H_2SO_4 mist (4,49,50). During September 1979 Southern Californians experienced "the worst ozone smog seige in 25 years" (21).

Previous acute (2-4 hour) exposure of human subjects to low levels of these pollutants (0.3 ppm O_3 and 98 $\mu g/m^3$, 0.14 μm H_2SO_4 aerosol), as separate exposures, produced no significant changes in pulmonary function. Single O_3 exposure to a slightly higher level of 0.4 ppm for 3 hours produced significant pulmonary function decrements. A combined exposure for 2 hours to 0.37 ppm O_3 and sulfur dioxide and 100 $\mu g/m^3$, 0.5 μm sulfuric acid aerosol showed small, statistically significant decrements in forced expiratory function (volumes and flows) in normal, healthy subjects (35). Statistical analysis of the group averaged data suggest that the mixture may have been slightly more irritating to the subjects than ozone exposure alone. Several investigators have shown that ozone appears to have the capability of producing a further decrease in pulmonary function with a second exposure (23,27,28,51). A pre-exposure to ozone might have the potential of magnifying the response to respirable sulfuric acid aerosol exposure. Both ozone and sulfuric acid aerosol are pulmonary irritants.

Based on the increased decrement in function with a second ozone exposure, this investigation was undertaken to determine if pre-exposure to 0.3 ppm O_3 would be capable of producing a significant decrement in pulmonary function following exposure to 100 $\mu g/m^3$, 0.13 μm H_2SO_4 aerosol. The test results of this study show this not to be the case, i.e. no significant decrement in function was observed with the H_2SO_4 exposure that followed the ozone exposure (third week).

The means of the individual subject differences in pulmonary function associated with the 0.3 ppm ozone exposures in this study were substantially smaller than those seen with a previous 0.4 ppm ozone exposure (23). Significant changes in pulmonary function were observed with the 0.4 ppm exposure. The results of these two studies in our laboratory suggest that the 0.3 ppm, 2-3 hour ozone exposure could represent an acute "threshold exposure"

level. However, in both studies, a single light-to-moderate exercise period of 100 W or 612 kg-m/min. was employed producing a $\dot{V}_E$ of approximately 35-40 L/min. Studies employing heavier exercise loading producing a $\dot{V}_E$ of 65-70 L/min could result in a significant pulmonary function decrement with exposure to 0.3 ppm O_3 for 2-4 hours.

Bronchial reactivity to methacholine showed no significant increase with any exposure. However, a substantial, almost significant decrease in bronchial reactivity occurred with the respirable H_2SO_4 aerosol exposure (second week). This is of interest, since an animal study presented at the American Thoracic Society meeting in Washington (May 1980) showed significant decreased bronchial reactivity to histamine following exposure to SO_2 gas. O'Cain et al. (52) exposed, via a chronic tracheostomy, eight dogs to 200 ppm SO_2 for 2 hours per day, 5 days per week for 3 months. Although this represents a chronic exposure to a high level of SO_2, this study has relevance to our research exposing human subjects to respirable H_2SO_4 aerosol. Atmospheric SO_2 gas is oxidized to sulfur trioxide, and with sufficient water vapor, the sulfur trioxide combines immediately with water to yield H_2SO_4 aerosol (1). Sulfuric acid has been shown to be more toxic than SO_2 in animal and human studies (5,6). O'Cain et al. showed decreased bronchial reactivity to histamine in dogs following SO_2 exposure; our study of human subjects demonstrated decreased bronchial reactivity to methacholine following respirable H_2SO_4 aerosol esposure.

This sequential exposure study demonstrated that the 0.3 ppm ozone pre-exposure did not push the subsequent H_2SO_4 aerosol exposure to significance. Employing a higher H_2SO_4 concentration is not appropriate in light of potential atmospheric levels. However, a larger sized H_2SO_4 aerosol might produce a significant effect, since use of larger mass median diameter particles should result in a larger percentage of the aerosol depositing in the upper and middle airways, where the deposition of ozone, and thus its irritant effect, is substantial (53). The target area of the lungs for the 0.13 μm aerosol, even with a threefold increase in diameter due to further hygroscopic growth following inhalation, is predominately in the pulmonary region (respiratory bronchioles and parenchyma - 54,55); whereas, the highly-reactive ozone molecules will probably affect the airways above the respiratory bronchioles. Deposition of both the ozone gas and H_2SO_4 aerosol at the same location in the airways might then produce a significant response. To further demonstrate probable differences in airway deposition sites of the gas and aerosol in this study, FEV_1 decreased with the ozone exposures on both the first and third weeks (Tables 5 and 7); while, it remained essentially unchanged for the H_2SO_4 exposure of the second week (Table 6). The functional test FEV_1 is thought to reflect primarily changes in the caliber of the larger airways (56,57,58).

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GLOSSARY

bronchial challenge: Physiological measurement of the bronchial reactivity to methacholine performed by inhaling successively increasing doses of methacholine. Bronchial reactivity to methacholine was expressed as the logarithm dose of methacholine which decreased the SGaw by 35% from control, called the log provocative dose (log PD_{35}).

dynamic compliance (C_L dyn): Volume change per unit of transpulmonary pressure minus the pressure of pulmonary resistance during airflow.

plethysmography: Body box method for determining airway resistance and static lung volumes.

pulmonary resistance (R_L): The sum of airway resistance and viscous tissue resistance.

quasistatic compliance (C_L stat): Measure of elastic recoil with no or insignificant airflow.

single breath nitrogen elimination rate (SBNER): Percentage rise in nitrogen fraction per unit of volume expired.